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Synthesis of α-PNA containing a functionalized triazine as nucleobase analogue

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ABSTRACT

The design of artificial structures such as Peptide Nucleic Acids (PNAs) capable of recognizing nucleic acids has attracted much attention. We report herein the design of L-homoserine derivatives bearing diaminotriazine groups as artificial nucleobase capable of pairing to thymine. We set up an original six-step synthetic route (45% overall yield) that enables the functionalization of the nucleobase analogue. Furthermore, we show that these modified amino acids can be incorporated, by solid-phase peptide synthesis, into alternate and homopolymer α-PNAs, thereby giving access to α-PNAs in which the nucleobase analogue bears functional groups that may prove useful for the multi-point recognition of nucleic acids.

Introduction

The design of functionalized ligands of biomolecules is of great interest for applications in health sciences.[1] On the one hand, the ability to tether molecular reporters without affecting the binding affinity of the ligand for its target may serve for sensing and imaging applications. On the other hand, the presence of side-groups that directly interact with the target through additional non-covalent interactions may be useful for enhancing the binding affinity and selectivity through multi-point recognition.[2] For instance, it has recently been shown that side groups present on glycopolymers have an effect on the recognition of lectins.[3]

In the field of oligonucleotide recognition, various chemical functionalizations,[4] or chemical modifications of the inter-nucleotidic linkage,[5] sugar moiety,[6] and nucleobase[5b,7] have been introduced into oligonucleotides to tune their stability and recognition properties for a specific application such as antisense technology.[1,8] In particular, functionalization of the nucleobase has been exploited for multi-point binding. For instance, functionalized cytosine analogues capable of 'clamp-like' binding through a combination of Watson–Crick pairing and hydrogen bonding on the Hoogsteen edge of the base pair[9] have been shown to confer enhanced potency to antisense phosphorothioate oligodeoxynucleotide.[10] More recently, it has been shown that the presence of additional side-groups that enhance π-stacking interactions greatly improves the selectivity of a novel nucleobase analogue for its complementary DNA partner.[11] It is therefore important, for promoting hybridization with nucleic acids, to develop synthetic methodologies that enable the construction of biomolecular conjugates featuring functionalized nucleobases tethered to an organic scaffold.

The instability in biological media and the poor cellular penetration properties of natural oligonucleotides have spurred interest in alternative artificial scaffolds that are capable of hybridizing with nucleic acids. Peptide-based scaffolds that interact with nucleic acids have attracted considerable interest, both for the study of prebiotic informational systems,[12] and for applications in biotechnology. Indeed, peptide-based scaffolds can be readily synthesized and further functionalized in a modular fashion. Peptide Nucleic Acids (PNAs) have been identified as an artificial class of peptide-based compounds that hybridize effectively with nucleic acids.[13] PNA feature a scaffold made of a repetition of *N*-(2-aminoethyl)-glycine moiety appended with nucleobases as side-chains. Functionalization of the scaffold has been shown to affect the stability of PNA–DNA and PNA–RNA duplexes.[14] Artificial nucleobases have also been placed onto PNA scaffolds. Cyanuryl groups have thus been proposed as thymine mimic,[15] while the insertion of 2-aminopyridine has been reported to lead to triplex formation with double-stranded RNA under physiological conditions.[16]

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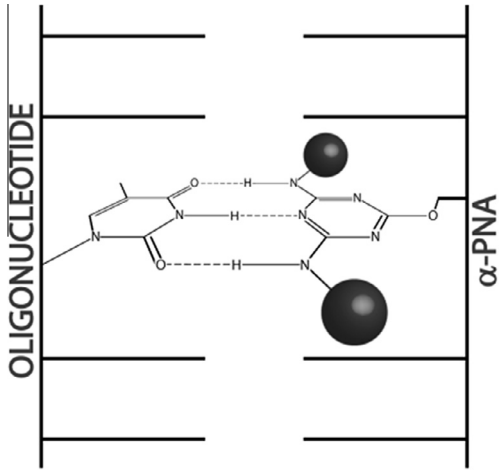

Scheme 1. Schematic representation of an α-PNA bearing a functionalized diaminotriazine moiety as a nucleobase analogue paired to thymine through hydrogen bonds. The spheres represent the functional groups that can be tethered onto the α-PNA scaffold and act as secondary binding groups for multi-point binding.

α-PNA is a class of PNAs which is made of α-amino acids, thereby giving a potential access to a larger scope of structures and sequences.[17] α-PNAs may form self-associated homoduplexes or heteroduplexes with DNA or RNA oligonucleotides. Eschenmoser et al. have recently demonstrated that α-PNAs bearing a non-canonical diaminotriazine nucleobase analogue effectively hybridize with oligothymine RNA by Watson–Crick-type pairing.[18] Following this discovery, α-PNAs have been functionalized with melamine for the triplex formation with dT_n DNA strands through bifacial recognition of thymine.[19] However, to date there are no examples of α-PNAs featuring functional groups on the nucleobase analogue that can act as secondary binding groups for multi-point binding. We report herein the design and synthesis of L-homoserine derivatives bearing functionalized *N*,*N*,*O*-triazines. We further demonstrate that these compounds can be readily used in solid-phase peptide synthesis to prepare alternate and homopolymer α-PNAs (Scheme 1).

Results and discussion

Design

Small molecules featuring a diaminotriazine moiety have been used to generate DNA-templated self-assembly[20] and novel nucleoside analogues featuring a diaminotriazine moiety as an artificial nucleobase have been reported.[21] Furthermore, triazines are popular synthons in organic and supramolecular chemistry,[22] and have already been assembled onto a variety of organic scaffolds.[23] While *N*,*N*,*N*-trisubstituted triazines such as melamine can lead to a supramolecular complex with two thymines,[19] our aim was to develop a functionalized nucleobase analogue that interacts with a single thymine through Watson-Crick-type interactions. Therefore, we selected *N*,*N*,*O*-triazines. Indeed, it has been shown that *N*,*N*,*O*-triazines may interact with complementary partners containing an imide group through three hydrogen bonds.[24] Novel nucleoside analogues based on *N*,*N*,*O*-triazines have then been developed.[25] Furthermore, *N*,*N*,*O*-triazines can be readily prepared by aromatic nucleophilic substitution starting from 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride).[26] Compared to the original method developed for preparing α-PNAs appended with diaminotriazine groups,[18] this synthetic strategy offers the unique advantage to further functionalize the diaminotriazine moiety in order to attach pendant groups which may present secondary binding groups (Fig. 1).

Since α-PNAs have been previously produced starting from either L-serine or L-homoserine,[17] we envisaged the synthesis of L-serine or L-homoserine derivatives bearing *N*,*N*,*O*-triazine side-groups as nucleobase analogues.

Synthesis

We first envisaged the use of L-serine. However, stability issues - attributed to an elimination reaction with concomitant production of dehydroalanine - were observed under the basic conditions that are typically used for substituting cyanuric chloride. L-homoserine was therefore selected instead of L-serine. The synthetic route we employed is described in Figure 2. L-Homoserine was successfully protected in two steps with carboxybenzyl (Cbz) and benzyl (Bn) groups to afford compound **1** in 89% overall yield. We found that

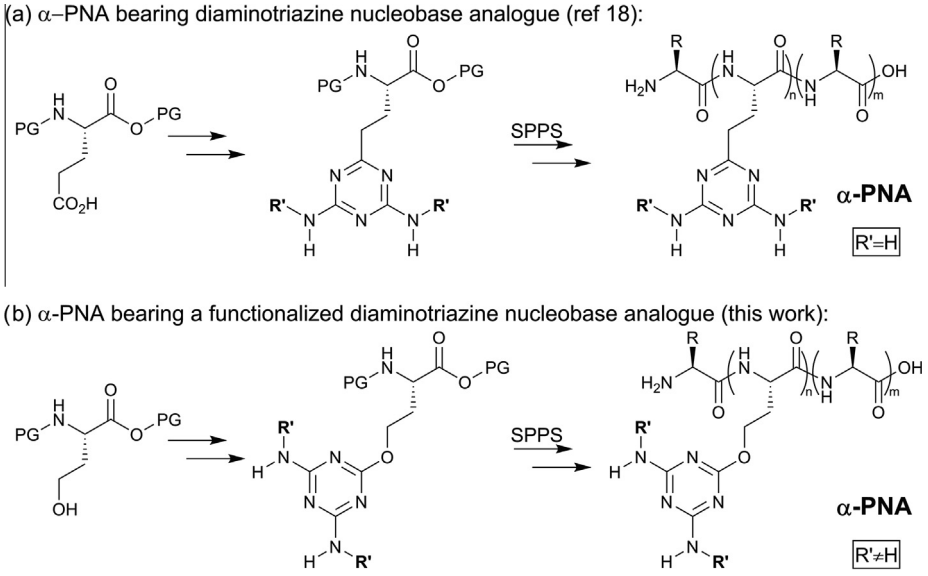

Figure 1. Synthetic routes previously used for preparing α-PNA bearing diaminotriazine and proposed strategy to access α-PNA bearing functionalized diaminotriazine nucleobase analogue. PG = Protecting group; SPPS = solid-phase peptide synthesis; R and R′ designate side groups.

it is crucial not to heat this compound above 30 °C—even during solvent removal—to avoid the formation of the corresponding lactone. A nucleophilic substitution reaction with cyanuric chloride was then carried out at low temperature to afford compound **2** in 79% yield. We found that it is critical to maintain the temperature at 0 °C for several hours as otherwise the isolated yield dropped to 26% when the reaction was immediately allowed to come back to room temperature. The dichlorotriazine compound **2** is a key building block from which derivatives can be prepared through the aromatic nucleophilic substitution of the two chlorines by primary amines. We focused our efforts on two compounds: one bearing unreactive aliphatic chains as a model compound and one bearing terminal amines which have the potential to be further functionalized (Fig. 2).

A nucleophilic substitution reaction between **2** and *n*-butylamine or *N*-tritylpentane-1,5-diamine afforded *N*,*N*,*O*-triazine compounds **3a** (95% isolated yield) and **3b** (87% isolated yield), respectively. In order to obtain compounds suitable for solid-phase peptide synthesis, we then removed Cbz and Bn protecting groups by a one-pot hydrogenolysis, and protected the amine with a Fluorenylmethyloxycarbonyl (Fmoc) group. The protection of the amino group was found to be more effective with *N*-(9-Fluorenylmethoxycarbonyloxy)succinimide (Fmoc-OSu, isolated yield 72-74%) than with *N*-(9-Fluorenylmethoxycarbonyloxy)-chloride (Fmoc-Cl, isolated yield 10–40%). Finally, the desired L-homoserine-based amino acids **4a** and **4b** bearing the functionalized diaminotriazine moieties were prepared in 6 steps with 43–47% overall yields following a divergent synthetic strategy that should enable the future preparation of derivatives.

Peptide synthesis

We then studied whether α-PNAs can be prepared from these novel modified amino acids. Amino acid **4a** was selected for preparing alternate (**Asp-Triaz**, **Ala-Triaz**) and homopolymer (**Triaz-Triaz**) peptides (Fig. 3). Alternate α-PNAs have been reported to adopt secondary structures that enable hybridization with complementary oligothymines.[18]

A manual solid-phase peptide synthesis using a 2-chlorotrityl chloride resin was employed and the stepwise yields were quantified by UV absorption spectroscopy after the Fmoc deprotection step.[27] The preparation of **Asp-Triaz** was first tested with standard procedures using DMF as the solvent and HBTU as the coupling agent. However, low stepwise coupling yields were observed (40–70%), especially during the first peptide couplings (Fig. 4,

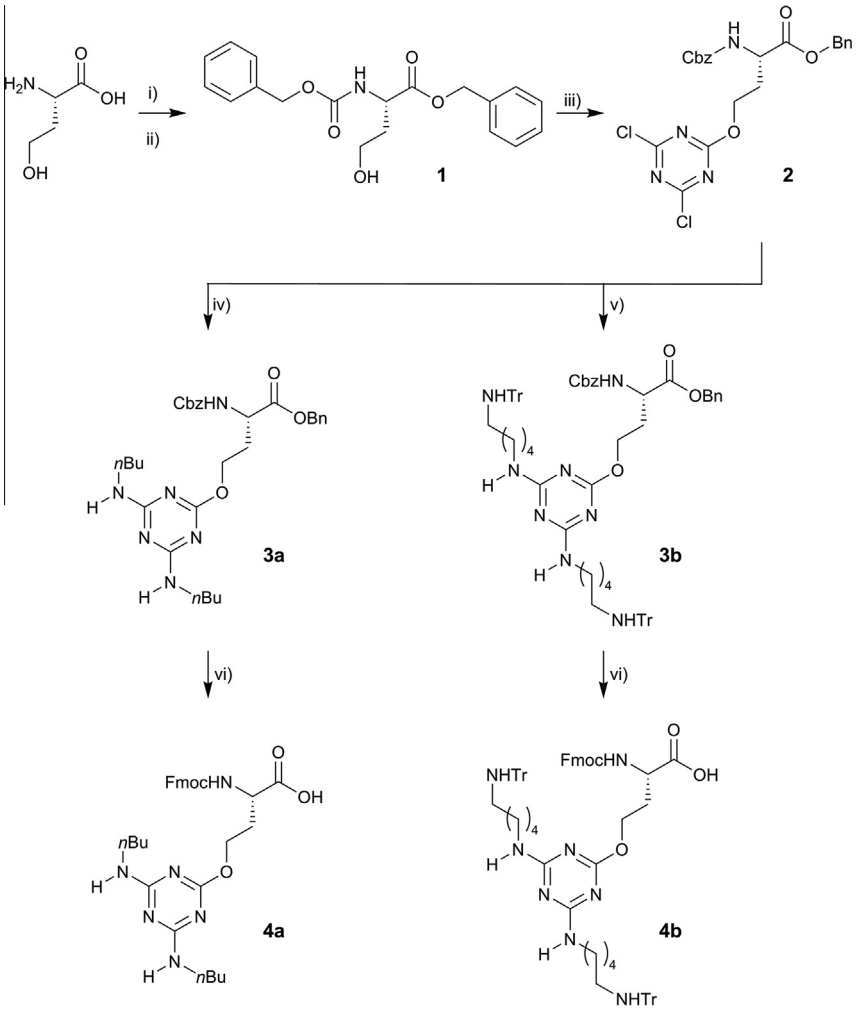

Figure 2. Synthesis of L-homoserine derivatives bearing functionalized diaminotriazine groups. (i) Benzyl chloroformate, $NaHCO_3$, 93%; (ii) benzyl bromide, DIPEA, DMF, 96%; (iii) cyanuric chloride, DIPEA, THF, 0 °C, 79%; (iv) *n*-butylamine, DIPEA, THF, reflux, 95%; (v) *N*-tritylpentane-1,5-diamine, DIPEA, THF, reflux, 87%; (vi) (a) Pd/C, H_2, 93–97% (b) *N*-(9-Fluorenylmethoxycarbonyloxy)succinimide, Na_2CO_3, 72–97%. *n*Bu = *n*-Butyl; Tr = Trityl; Cbz = Carboxybenzyl, Bn = Benzyl; Fmoc = Fluorenylmethyloxycarbonyl ; DIPEA = Diisopropylethylamine.

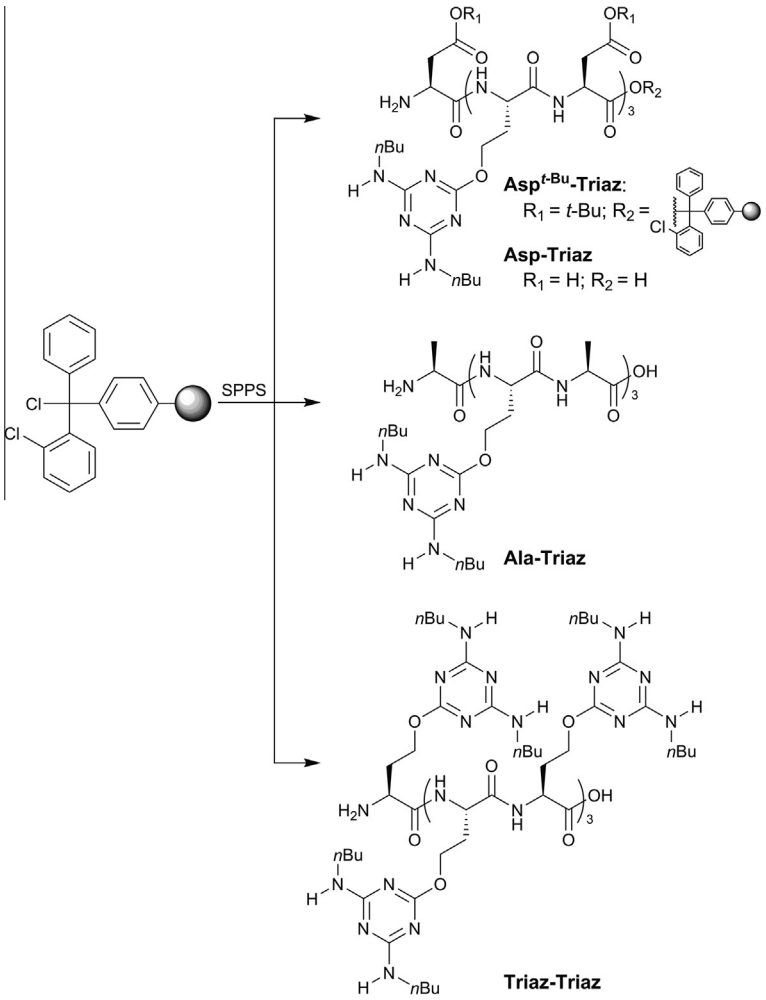

Figure 3. Synthesis of alternate (**Asp-Triaz**, **Ala-Triaz**) and homopolymer (**Triaz-Triaz**) α-PNAs by solid-phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin.

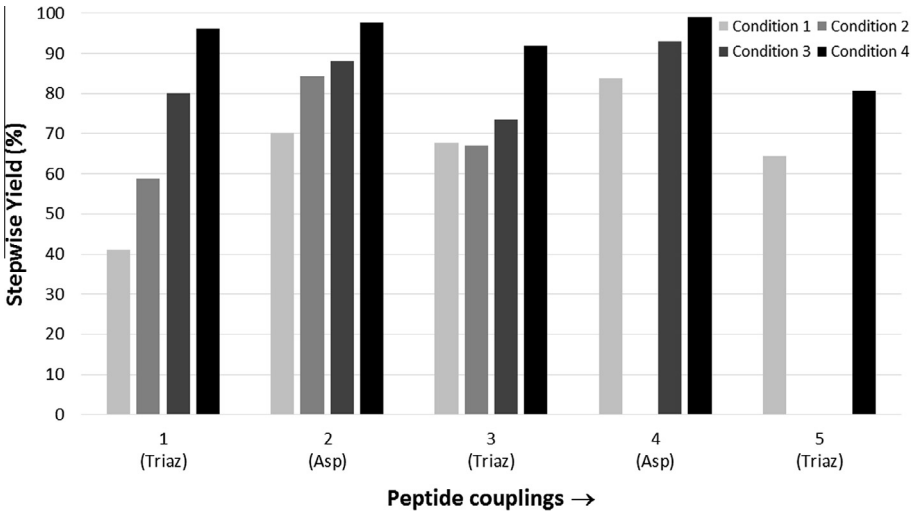

Figure 4. Optimization of the peptide synthesis procedure for preparing alternate α-PNA **Asp-Triaz**. Condition 1: HBTU, DMF; condition 2: HBTU, DMF, pre-activation and double coupling; condition 3: HATU, DMF, pre-activation and double coupling; condition 4: HATU, NMP/DMSO, pre-activation and double coupling.

condition 1). Amino acid pre-activation and double coupling induced a slight but unsufficient improvement of the coupling yields (Fig. 4, condition 2). Gratifyingly, replacing HBTU with the more reactive HATU induced a significant increase in the coupling yields, reaching 70–80% (Fig. 4, condition 3). Finally, DMF was replaced with a mixture of NMP/DMSO and stepwise coupling yields greater than 95% could be achieved (Fig. 4, condition 4). This condition enabled to maintain a good overall yield of 70% after five coupling steps whereas only 10% was reached with the initial condition.

Using this optimized condition, we set up the preparation of α-PNAs **Asp-Triaz**, **Ala-Triaz**, and **Triaz-Triaz** and monitored the peptide couplings by UV absorption spectroscopy after the Fmoc deprotection steps. However, although good stepwise yields were obtained during the preparation of 7-mer alternate α-PNA **Asp-Triaz**, the deprotection and cleavage step converting **Asp$^{t\text{-Bu}}$-Triaz** into **Asp-Triaz** proved troublesome. Indeed, although the desired product was detected by LC–MS analysis, a rapid degradation was observed during this treatment in TFA/DCM. The proportions of TFA, the nature of the acid (Formic acid vs TFA), the temperature (rt vs 0 °C), and the reaction time were carefully tested but, unfortunately, did not provide a reliable solution and **Asp-Triaz** could not be obtained. This issue may originate from aspartimide formation which is well-known for peptide sequences rich in Asp.[28] In line with this hypothesis, we found that 7-mer alternate α-PNA **Ala-Triaz** and homopolymer α-PNA **Triaz-Triaz** were much more robust to the acidic cleavage conditions. Thus, the products were cleaved from the resin by acidic treatment (TFA/DCM) and purified by reverse-phase HPLC. α-PNAs **Ala-Triaz**, and **Triaz-Triaz** were isolated in 10% and 28% yields, respectively.

Conclusions

We reported herein the design and synthesis of novel L-homoserine derivatives **4a** and **4b** bearing functionalized *N*,*N*,*O*-triazine groups. These compounds were successfully prepared in 6 steps with a good overall yield (45%). We demonstrated that this synthetic strategy enables tethering functional groups onto the triazine core. Furthermore, we showed, as a proof-of-concept, that the modified amino acid **4a** can be incorporated into alternate (**Ala-Triaz**) and homopolymer (**Triaz-Triaz**) peptides. These novel α-PNAs bearing functionalized nucleobase analogues may prove useful for hybridizing to nucleic acids through multi-point recognition.

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Supplementary data

Supplementary data (experimental procedures and characterization data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.03.072.

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